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(71) Applicants: THE HOSPITAL FOR SICK CHILDREN [CA/CA]; 555 University Avenue, Toronto, Ontario M5G 1X8 (CA). SPECTRAL DIAGNOSTICS INC. [CA/CA]; 135 - Unit 2, The West Mall, Toronto, Ontario M9C 1C2 (CA).

(72) Inventors: COLES, John, G.; 162 Alexandra Boulevard, Toronto, Ontario M4R 1M4 (CA). TAKAHASHI, Miyoko; 65 Franklin Avenue, North York, Ontario M2N 1B8 (CA). YOUNG, David, S., F.; 51 Baldwin Street #3, Toronto, Ontario M5T 1L1 (CA). BROCKHAUSEN, Inka; 86 Lenty Avenue, Toronto, Ontario M4E 4R4 (US).

(74) Agent: GOWLING, STRATHY & HENDERSON; 160 Elgin Street, Ottawa, Ontario K1P 1C3 (CA).

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(54) Title: INHIBITION OF HUMAN XENOGENIC OR ALLOGENIC ANTIBODIES TO REDUCE XENOGRAFT OR ALLOGRAFT REJECTION IN HUMAN RECIPIENTS

(57) Abstract

Reactivity between an alloantigen and an anti-alloantigen is indicative of immunological reactivity between two biological samples of the same species. Reactivity between a xenoantigen and an anti-xenoantigen is indicative of immunological reactivity between two biological samples of different species. In many cases both of the reactions are indicative of an antibody-mediated rejection. Anti-antibodies can be employed to reduce cross-reactivity in many transplantation-type situations, either within a similar species, or across species lines. These anti-antibodies are prepared against the antibodies responsible for the antibody-mediated rejection. These anti-antibodies can then be used *in vivo* or *in vitro* to complex with the antibodies thus reducing or eliminating reactivity between an alloantigen and an anti-alloantigen or reactivity between a xenoantigen and an anti-xenoantigen between any two species combinations. These antibodies may also be used to target and eliminate the B-cells that produce anti-xenogenic antibodies.

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# INHIBITION OF HUMAN XENOGENIC OR ALLOGENIC ANTIBODIES TO REDUCE XENOGRAFT OR ALLOGRAFT REJECTION IN HUMAN RECIPIENTS

## Field of the Invention

This invention is directed towards anti-idiotypic antibodies which can be used for reducing allograft or xenograft rejections. This invention is also directed to methods for preparing these anti-idiotypic antibodies and methods of using these antibodies to reduce or eliminate reactivity between a xenoantigen and an anti-xenoantigen between any two species combinations, or to eliminate the B-cells that produce anti-xenogenic antibodies, or to reduce or eliminate reactivity between allogenic antigen and an anti-alloantigen. These antibodies can also be used to measure or label its cognate antibody and /or the cells that produce them and may be applied diagnostically.

## Background and Prior Art

In many transplantation situations, within species, there is concern for differences between the allotype, especially the HLA type, of a cell donor and a cell recipient. In situations where allogenic cells or tissues are taken from a donor and introduced into a recipient, it is desirable that the donor and recipient be as closely HLA matched as possible. The presence in the patient's serum of antibodies against HLA antigens of the donor (donor specific cross-match), or against a high percentage of HLA alleles (PRA testing) predicts a high risk of graft rejection.

An alloantigen is a direct or indirect product of an allele which may be detected as an antigen by another member of the same species. The products of such alleles include encoded polypeptides, but also specific polysaccharides and lipids synthesized by allele encoded enzymes. Alloantigens of particular interest in the present invention include

histocompatibility antigens, blood group antigens such as the ABO, Lewis group, the endothelial alloantigen system, and the like. Of special interest are histocompatibility antigens which include major, known as HLA in humans, and minor histocompatibility antigen groups. Anti-alloantigen are molecules which are capable of reacting with, or preferentially associating with, an alloantigen. Examples of such anti-alloantigens include anti-allotypic immunoglobulins or fragments thereof, anti-allotypic T-cell receptor or derivatives or fragments thereof, HLA binding peptides, etc., and combinations thereof.

Shortage of human organs is a major limitation to application of transplantation for end-stage organ disease. This has stimulated a strong interest in xenogenic transplantation. The pig has been considered by many investigators to be a suitable organ donor for human transplantation. Porcine to human xenotransplantation, however, is complicated by hyperacute rejection initiated by non-elicited human antibodies, referred to as preformed antibodies, binding to porcine xenoantigens, for example, porcine aortic endothelial cells (PAEC) xenoantigens. Other species that have been targeted for possible xenogenic transplantation into humans include sheep, goats and non-human primates.

In both examples discussed above, the problem of antibody-mediated rejection can be eliminated or reduced in severity by the use of the anti-antibodies of the present invention.

The prior art describes some success in facilitating non-xenotransplants between ABO-mismatched individuals. In human to human transplantation, the extracorporeal removal of naturally occurring anti-A and/or anti-B antibodies using a method similar to those described in several references (U.S. Patents 4,137,401, 4,238,473; U.K. Patent 1544908; and European Patent Application 89311540.2) has enabled successful transplantation of kidneys and bone marrow between ABO mismatched

individuals (Bannett, A.D., McAlack, R.P., Raja, R., Baquero, A., Morris, M.: Transplant. Proc. XIX: 4543-4546, 1987 and Bensinger, W.I., Buckner, C.D., Thomas, E.D., Clift, R.A.: Transplantation 33: 427-429, 1982).

PCT Application WO 93/03735 describes the use of at least one carbohydrate xenoantigen which is capable of binding one or more antibodies involved in a antibody-mediated xenograft rejection. The carbohydrate xenoantigen of the prior art can be used to inhibit xenoantibodies *in vitro* or *in vivo*.

In a further example, PCT Application WO 93/16729, provides an anti-human IgM antibody to lower the levels of natural antibodies which react with a xenograft in a patient who has, or is about to receive, a xenograft. In particular, this prior art discloses the production of anti-human IgM antibodies which react with the $\mu$ chain portion of the constant region of human antibodies, with such $\mu$ chain being characteristic of an IgM antibody.

The proposals of the prior art are limiting in their application as they are either directed towards removal of antibodies against identified carbohydrate antigens, or to the complete and non-specific removal of all IgM antibodies. In contrast, the present invention has adopted an approach for the removal or attenuation of anti-alloantigen antibodies or anti-xenoantigen antibodies, which is more inclusive since it can be effective against all classes of antibodies, regardless of isotype. Furthermore, the present approach is more specific since it involves depletion of only the offending alloreactive or xenoreactive antibodies, while preserving the vast majority of the total antibody complement, and thus permitting maintenance of normal immunological surveillance against infection and oncogenesis.

Geller et al. (Transplantation, Vol. 55, pp. 168-172, 1983) developed a series of hybridoma-derived monoclonal antibodies specific for

the polyreactive human monoclonal antibody 103, which they had previously shown to bind efficiently to porcine endothelial cells. Their studies, however, showed that the idiotypic reagents used to block binding of monoclonal antibody 103 to endothelial cells did not show more than a 30% inhibition. They concluded by suggesting that the anti-idiotypic reagents recognized structures outside of the paratope (antigen-binding site of the mAb 103). In contrast to this prior art, the present application utilizes affinity-purified antibodies derived from human serum reactive with pig EC. Thus in the present invention, the production of murine anti-idiotypic antibodies would be based on the complete array of antibodies, representing a mixture of monoclonal specificities, which functionally react with pig EC. This would be expected to constitute a therapeutically more inclusive approach.

Summary of the Invention

According to the present invention, there is provided anti-idiotypic antibodies recognizing a limited repertoire of idiotypic specificities, which recognize specific cognate antigens. These antigens can be alloantigens or xenoantigens, thereby leading to the xenogenic or allogenic reaction response in xenografts or allografts.

In one embodiment of the present invention there is provided a ß-type anti-idiotypic antibody selected from the group consisting of a ß-type anti-idiotypic antibody to a human anti-xenoantigen antibody, and mixtures thereof, and a ß-type anti-idiotypic antibody to a human anti-alloantigen antibody, and mixtures thereof.

In another embodiment of the present invention there is provided a method for reducing or preventing graft rejection in a patient comprising contacting an effective amount of at least one ß-type anti-idiotypic antibody selected from the group consisting of a ß-type anti-idiotypic antibody to a human anti-xenoantigen antibody, or mixtures thereof, and a ß-type anti-idiotypic antibody to a human anti-alloantigen antibody, or mixtures thereof,

to reduce blood levels of said human anti-alloantigen or human anti-xenoantigen antibodies and the B-cells that produce said antibodies, in a patient in need of such reduction.

In a further embodiment, a method is provided for measuring or labelling a cognate antibody or the cells that produce said antibody using a ß-type anti-idiotypic antibody selected from the group consisting of a ß-type anti-idiotypic antibody to a human anti-xenoantigen antibody, and a ß-type anti-idiotypic antibody to a human anti-alloantigen antibody, wherein said cognate antibody to be measured corresponds to said ß-type anti-idiotypic antibody.

The present invention also provides a method for reducing or preventing graft rejection in a patient comprising contacting an effective amount of at least one antibody, or mixtures thereof, reactive against a xenoantigen, such that the xenoantigen is modified to reduce or prevent the binding of said xenoantigen to a human anti-xenoantigen antibody.

In another embodiment of the present invention there is provided a method for reducing or preventing graft rejection in a patient comprising contacting an effective amount of at least one peptide sequence homologous to a ß-type anti-idiotypic antibody selected from the group consisting of a ß-type anti-idiotypic antibody to a human anti-xenoantigen antibody, or mixtures thereof, and a ß-type anti-idiotypic antibody to a human anti-alloantigen antibody, or mixtures thereof, to reduce or prevent the binding of said xenoantigen or alloantigen to a human anti-xenoantigen or human anti-alloantigen antibody.

Brief Description of Drawings

Figure 1 shows porcine aortic endothelial cell (PAEC) lysis after incubation with either: phosphate buffered saline, human complement (C), non-xenogeneic monoclonal human IgM+C, XeIgM+C, human AB plasma, and human plasma (HuPl) preincubated with xenoantigen (XeAg).

Figure 2 shows the effect of mouse sera following immunization with human-xenoreactive IgM (XeIgM) and Troponin-T (control) on binding of human IgM to PAEC.

Detailed Description of the Invention

The present invention addresses the problem of antibody-mediated rejection in the rejection of an alloantigen or xenoantigen following organ or tissue transplant within species or outside of species. The overall concept of the present invention is to prepare antibodies, either monoclonal or polyclonal, which will complex with either non-elicited or elicited human antibodies reactive against a xenoantigen or an alloantigen.

In the case of a transplant within species, the presence in the recipient's serum of antibodies against HLA antigens of the donor, will result in graft rejection. Examples of such anti-alloantigens include anti-allotypic immunoglobulins or fragments thereof, anti-allotypic T-cell receptor or derivatives or fragments thereof, HLA binding peptides and combinations thereof.

For transplantation outside of species lines, it has been found for example that porcine to human xenotransplantation is complicated by hyperacute rejection initiated by non-elicited human antibody, primarily IgM, binding to porcine aortic endothelial cell (PAEC) xenoantigens. In one embodiment of the present invention, anti-idiotypic antibodies to human xenogenic IgM may limit hyperacute rejection. This approach can also be

used against all classes of xenoreactive antibodies against the xenoantigens of any species regardless of isotype including IgM, IgG, IgA, and IgE. Xenoreactive IgM against porcine xenoantigens is exemplified in the present invention, but is not to be construed as limiting. Other species that have been targeted for possible xenogenic transplantation include sheep, goats and non-human primates. The present invention can also be used to prepare ß-type anti-idiotypic antibodies to a human anti-xenoantigen antibody against xenoantigens from other species.

The ß-type anti-idiotypic anti-human antibody can be used according to the present invention to reduce blood levels of xenoreactive or alloreactive antibodies and the B-cells that produce them. Such reduction may be accomplished by contacting whole blood or serum of a human patient with the anti-idiotypic anti-human antibodies in either an *in vivo* or *in vitro* method. In some embodiments of the present invention, it would be useful to use a mixture of different anti-idiotypic anti-human antibodies to react with a range of different xenoreactive or alloreactive antibodies.

In one example of the present invention, in an *in vivo* procedure, the antibodies would be administered to a patient in an amount effective to reduce blood levels of the preformed human xenogenic antibodies, or xenoantibody producing B-cells, thus reducing or eliminating xenograft rejection.

According to this *in vivo* method, the anti-idiotypic anti-human antibody is administered in a pharmaceutically acceptable carrier. As representative examples of such carrier, there may be mentioned normal saline solution, buffers, etc. Such pharmaceutical carriers are well known in the art, and the selection of suitable carriers is deemed to be within of the scope of those skilled in the art from the teachings contained therein.

The anti-idiotypic anti-human antibody may be administered, for example, intravenously or intramuscularly.

In general, to inhibit or reduce xenograft rejection, the antibody according to the present invention, can be administered in an amount effective to reduce blood levels of the preformed human xenogenic antibodies, or xenoantibody producing B-cells. The treatment would preferably start at or immediately prior to the transplantation and would continue, as required.

Several recent papers have emphasized the improvement in fusion stability using immortalized human lymphocytes for the production of monoclonal antibodies using both conventional methods and clones propagated in bioreactors. The production of monoclonal Abs derived from human cell lines obviates the potential adverse HAMA response and would facilitate the regulatory process required for approval of therapeutic agents. This approach could be used to produce the human anti-human antibodies of the present invention, to eliminate any potential human anti-mouse response.

Recently described techniques of phage antibody production have allowed the manufacture in prokaryotic systems of completely human antibodies. Repertoires of antibody genes are cloned into phage, which then display functional antibody fragments on their surface and provide an efficient means for antibody selection on exposure to specific antigens. This approach could likewise be used to produce anti-idiotypic anti-human antibodies by using, for example, the xenoreactive antibody or alternatively, the xenoantigens, isolated by the methods described in this invention, as specific ligands to select recombinant antibody fragments conveyed on the surface of bacteriophage particles, according to the principles described for this bacteriophage-based screening system.

In an *in vitro* method, the antibodies of the present invention would be contacted with the blood or serum derived from a patient and after such treatment, the treated blood would be returned to the patient (extracorporeal circulation). Thus, for example, anti-idiotypic anti-human antibody may be supported on a suitable solid support and blood or serum derived from a patient is contacted with the supported antibody and returned to the patient.

Any one of a wide variety of solid supports may be employed for supporting the antibody in such *in vitro* treatment. Thus, for example, the support may be in the form of beads in a column, or a solid sheet or the like. Such techniques are generally known in the art and should be apparent to those skilled in the art from the teaching herein.

The method of the present invention for reducing blood levels of anti-xenogen antibodies or anti-allogen antibodies can be used in conjunction with other techniques to reduce or eliminate graft rejection as are known in the art.

The ß-type anti-idiotypic antibodies of the present invention can also be used to measure or label its conjugate antibody and/or the cells that produce them, in a diagnostic method. For example, the anti-idiotypic antibodies of the present invention can be used as a reagent to measure the titre of the xenogenic antibody in patients preceding or following transplantation, including a xenogenic organ or tissue. Furthermore, the ß-type anti-idiotypic antibodies of the present invention could be used to measure the amount of xenoreactive antibodies deposited in biosamples from transplanted grafts. In addition, the ß-type anti-idiotypic antibodies of the present invention can be used to label T-cells or B-cells, producing the xenogenic antibodies, using FACS (Fluorescent-Activated Cell Sorting).

The antibodies generated against antigens, such as the porcine xenoantigens described in this method, can be used to reduce reactivity between xenoantigens and xenoantibodies. In this example, anti-porcine endothelial cell antigen antibodies may be used to reduce reactivity between xenoantigens and xenoantibodies. This may be accomplished by exposure of the monoclonal antibody to xenoantigens present on xenoantigen containing cells, either in its unmodified form or modified to render it non-complementing fixing. Such exposure and binding of the monoclonal antibodies would have the effect of modulating a reduction in antigenicity. Alternatively, these antibodies may compete with the native xenogenic antibodies for binding sites to minimize the effect of the native xenogenic antibodies.

ß-type anti-idiotypic antibodies are the mirror image of their complementary antibody (AB1) and thus are topologically like the antigen that produced AB1. The peptide sequences of the monoclonal anti-idiotypic antibodies may be obtained using current peptide sequencing technology (e.g. automatic sequencers may be used to sequence overlapping peptide segments of the anti-idiotypic antibodies). The peptide sequences of the anti-idiotypic antibodies may also be derived from its DNA sequence. This may be done by constructing overlapping oligonucleotide primers to VDJ or VJ sequences of the rearranged heavy chain and light chains from cDNA libraries of the hybridomas that produce the anti-idiotypic antibodies and using PCR techniques to increase the amount of DNA needed for sequencing those genes. The DNA sequences may be expressed in an appropriate system to produce peptides or glycopeptides for use as competitive inhibitors of xenogenic antibody binding. Alternatively, peptide sequences may be chemically synthesized using currently available chemical techniques. Thus, the peptides could be used to inhibit the binding of the human xenogenic antibodies to the xenoantigens, thus reducing antibody mediated rejection.

Although the methods described herein will be described in particular detail with regard to the preparation of an anti-human IgM antibody reactive against antibodies which react with antigens on porcine aortic endothelial cells, the invention should not be construed as so limiting.

Two approaches were used for the preparation of antibodies reactive against human xenogenic IgMs (XeIgM). In one method, isolated xenogenic IgM from human plasma was used to immunize Balb/c mice for the production of murine anti-XeIgM. The second method involved the use of fixed porcine aortic endothelial cells as antigen. In this method, resulting mouse antibodies will react with the same epitope as the human xenogenic IgM. These murine antibodies are then used to inject a syngeneic mouse. The second mouse will not recognize the common component of the antibody as foreign, but will only recognize the antigen-binding site as an antigen. The antibodies from the second mouse will more effectively produce anti-idiotypic anti-human XeIgM. These two examples will be discussed in further detail below.

In the first protocol, human immunoglobulins were used to raise murine monoclonal antibodies which will bind the human immunoglobulin. In one embodiment of the present invention, the human immunoglobulins were naturally occurring xenoreactive antibodies, present in human blood which specifically bind to the antigens present on pig aortic endothelial cells (PAEC) and mediate the lysis of these cells. All isotypes including IgM, IgG, IgA, and IgE, will be included within the class of the isolated xenoreactive antibodies. In one embodiment of the present invention, xenoreactive IgM was affinity isolated using anti-$\mu$ membrane affinity chromatography with glycine elution. Before using the xenoreactive IgMs of the present invention as an antigen for the production of mouse antibodies specific against the xenoreactive IgMs, the IgMs were tested to ensure that they were xenoreactive against PAEC antigens. In addition, the XeIgMs were used to affinity isolate the xenoantigens from cultured porcine aortic endothelial cells.

The authenticity of the isolated glycoproteins as xenogenic structures were confirmed by demonstrating their ability to functionally interact with and competitively inhibit human AB plasma mediated lysis of PAEC.

As mentioned previously, the purified human xenoreactive antibodies, specifically PAEC reactive IgM, were injected into Balb/c mice. Immune splenocytes were obtained from the mice and fused with SP2/0 cells to establish hybridoma lines which produce ß-type anti-idiotypic monoclonal antibodies. Basic techniques for the preparation and purification of antibodies are disclosed in "Basic Principles of Antigen-Antibody Reactions", Elvin A. Kabat, Methods in Enzymology, Vol. 70, (1980), pp. 3-70, including the procedure for the production of monoclonal antibodies which are described by G. Kohler and C. Milstein, in Nature (London), Vol. 256, (1975), p. 495, and Eur. J. Immunol. (1976), 6:511-519; all of which are incorporated herein by reference. Briefly, fused cell in selective medium containing hypoxanthine, aminopterin, and thymidine were added to 300-5000 wells of tissue culture plates, which were preseeded with feeder cells. Hybridoma cultures were subcloned 2-3 times by limiting dilution method on a feeder layer of 1-3x$10^4$ mouse peritoneal macrophages. Monoclonal or polyclonal antibodies can be prepared according to the present invention, although monoclonals are exemplified. Anti-idiotypic antibodies can be prepared from the serum of animals such as rabbits, horses, or goats, which have been immunized against the appropriate antigens (i.e. immunoglobulins).

Immunoglobulins contain antigen-combining sites that determine the binding specificity of the antibody and are themselves immunogenic. These serologically defined epitopes are described as idiotypes. The collection of idiotopes on an immunoglobulin make up its idiotype and antibodies elicited against them are referred to as anti-idiotypic antibodies. The humoral response to a syngeneic immunoglobulin contains anti-idiotypic antibodies that fall into two classes, those recognizing idiotopes

that lie within the antigen binding site of the first immunoglobulin (ß anti-idiotypes) and those recognizing idiotopes that lie outside this region (α anti-idiotypes or anti-framework antibodies). The humoral response to a non-syngeneic immunoglobulin will also include antibodies against allotypic determinants which complicate the screening of hybridoma culture supernatants for the presence of anti-idiotypic antibodies. Therefore, it is necessary to remove mouse anti-human allotypic antibodies prior to screening for anti-idiotypic antibodies.

In the second procedure, fixed whole PAECs were injected into Balb/c mice for an *in vivo* immunization. In addition, *in vitro* immunizations were done by co-culturing non-immunized splenocytes with fixed PAEC. Both were fused to SP2/0 myeloma partners. The resulting monoclonal antibodies inhibit the binding of human xenogenic IgMs to PAEC, demonstrating that the monoclonal antibodies bind the same epitopes that are recognized by the human xenogenic IgM. As mentioned previously these monoclonal antibodies can be used directly to inhibit or reduce the binding of human xenogenic IgMs to the corresponding xenoantigen, thus reducing xenograft rejection.

The mouse monoclonal antibodies, produced using this second procedure, were then used as an antigen to immunize a syngeneic mice strain. The use of inbred mouse strains will therefore avoid the allotype response and will facilitate the production of anti-idiotypic antibodies. The resulting hybridoma cultures are screened for ß-type anti-idiotypic activity.

The following examples are offered by way of illustration and not by limitation.

# INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/CA 95/00337

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| WO-A-9421799 | 29-09-94 | AU-B- | 6279294 | 11-10-94 |

# INTERNATIONAL SEARCH REPORT

Interr ial Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/42 A61K39/395 G01N33/577 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | LA PRESSE MEDICALE, vol. 21, no. 41, 2 December 1992 PARIS, FRANCE, pages 1932-1938, L. JEFFREY ET AL. 'Progrès récents en xénotransplantation.' see page 1933, left column, line 36 - page 1934, left column, line 18 --- | 1-5, 9-13,16 |
| X | TRANSPLANTATION, vol. 55, no. 1, January 1993 BALTIMORE, MD, USA, pages 168-172, R. GELLER ET AL. 'Evidence that polyreactive antibodies are deposited in rejected discordant xenografts.' cited in the application see the whole document --- | 1-5,12, 13 |

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[X] Further documents are listed in the continuation of box C.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WO,A,94 21799 (AUSTIN RESEARCH INSTITUTE) 29 September 1994<br>see page 4, line 3 - page 5, line 19<br>see page 25, line 16 - page 26, line 19<br>see claims 9,10 | 1-5, 9-13,16 |
| P,X | CIRCULATION, vol. 90, no. 4 part 2, October 1994 NEW YORK, NY, USA, page I419<br>D. YOUNG ET AL. 'Inhibition of human xenogeneic antibody binding to porcine aortic endothelial cells by mouse anti-idiotypic antibodies.'<br>see abstract 2251 | 1-5,9, 10,13,16 |
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# INTERNATIONAL SEARCH REPORT

| l national application No. |
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**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Please see annex!

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

REMARK: Although claims 9,10,11 (the latter as far as in vivo method is concerned), 15 and 16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.